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Genetic Transformation: a Retrospective Appreciation

First Griffith Memorial Lecture

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Exegi monumentum aere perennius (Horace, *Odes*, III, xxx, 1).

(I have completed a monument more lasting than brass.)

On the night of 17 April 1941, almost exactly 25 years ago, Fred Griffith and his colleague, W. M. Scott, were killed by a German bomb during an air raid on London. At the time of his death Griffith was about 60 years old. In an obituary written shortly afterwards it was suggested that a fitting memorial to these two men would be the construction of a new Ministry of Health building more worthy of Griffith and Scott, and of the dedicated and important epidemiological research which they had done within the dilapidated environment of their old laboratory. No one then guessed that Griffith had already built his own memorial 13 years previously when, in 1928, he published in the *Journal of Hygiene* his famous and remarkable paper on the significance of pneumococcal types (Griffith, 1928). This evening, in this first Griffith Memorial Lecture, it is my privilege and intention to try to revive for you the essence of Griffith's most outstanding discovery and, in so far as I can, to present it in perspective against the somewhat sophisticated and mature background of modern molecular genetics to which it gave birth.

Fred Griffith has been described as a shy and reticent man, whose quiet kindly manner, and his devotion to his job, made him a lovable personality to those few who got to know him. Outside his work he found his pleasure in ski-ing and in walking on the Sussex downs where he had built a cottage. Like his elder brother Stanley, who died only a few days before him, he was a medical bacteriologist whose primary and abiding interest, and his life's work, was the epidemiology of infectious disease. He believed that a proper understanding of epidemiological problems could come only from more detailed and discriminating knowledge of infectious bacterial species, and of the nature of bacterial virulence and variation. For a time he worked on the typing of tubercle bacilli with Stanley Griffith, whose published work on this topic extended over many years and was prolific. On the contrary, Fred Griffith's output of scientific papers was, by comparison, remarkable for its paucity. In view of the quality and distinction of what he did publish, however, I think that this must be ascribed to an inate humility and capacity for self-criticism so that he offered to posterity only those products of his research which he judged to be new and important.

I suppose that Griffith would have deemed his most valuable contribution to epidemiology to be the discovery that many serological types exist within group A streptococci; these are the causative organisms of what were, at that time, such

prevalent and lethal human infectious diseases as puerperal fever, erysipelas and scarlet fever, not to mention acute tonsillitis and its complications such as rheumatic fever and middle ear disease—now so admirably controllable by penicillin to which these bacteria do not develop resistance. For us, of course, as for all biologists, Griffith's continuing fame rests on his discovery of the transformation of pneumococcal types. If you were now to ask any microbial geneticist or molecular biologist, who knew nothing of epidemiology, 'What happened in 1928?,' the odds are that he would at once reply, 'Well, for one thing, Griffith discovered transformation'.

The story of this discovery is told in his 1928 paper, which is the only one he wrote on this topic. Pneumococci are divisible into a number of well-defined types according to the serological specificity of the polysaccharide capsule which they possess. At the same time, the virulence of *all* pneumococcal types is determined by their capsulation which protects the invading bacteria from phagocytosis. Among Griffith's most significant discoveries was the observation, which was quite novel at the time, that more or less stable, non-capsulated and avirulent variant strains could be induced by the growth of capsulated pneumococci in the presence of type-specific antiserum. The first half of the 1928 paper concerns the stability of these avirulent variants. Griffith observed that inoculation of mice with large doses of some of these variants very occasionally produced a lethal infection from which virulent capsulated bacteria were recovered. He thought that this reversion to virulence might be due to the fact that the avirulent bacteria had not entirely lost the capacity to synthesize capsular polysaccharide so that, in dense populations such as were injected, a sufficient concentration of polysaccharide might have been present to restore some kind of autocatalytic process which led to normal capsular synthesis.

If this were so, then it should be possible to revert stable non-capsulated strains to virulence by providing them with exogenous capsular material. To test this idea, Griffith inoculated mice subcutaneously with a mixture of small numbers of living avirulent bacteria, and dense suspensions of heat-killed virulent organisms, neither of which yielded virulent capsulated bacteria when injected alone. He found that mice which received the mixtures frequently died from septicaemia and that capsulated virulent organisms could be isolated from their blood. He gave the name 'transformation' to this phenomenon and, in the field of bacterial genetics at least, this name is still used specifically to describe it.

Griffith found that transformation occurred most frequently when the avirulent bacteria originated from the same capsular type as the heat-killed transforming bacteria. However, the main interest of the phenomenon, both at the time and subsequently, centred on the discovery that avirulent pneumococci originating from one capsular type (say, type II) could be permanently transmuted to another type (say, type I or III) corresponding to that of the heat-killed capsulated bacteria with which they were inoculated into mice. For Griffith, as for all medical bacteriologists both then and for many years afterwards, the interest and importance of transformation lay in the light it shed on the nature of virulence and on such epidemiological problems as the stability of serological types and variations in the incidence of type infections. From these points of view the demonstration that both the type and the virulence of well-defined epidemiological varieties of bacteria

could be specifically altered at will, could hardly have been more dramatic. In fact, Griffith appears to have hesitated for some time before publishing his finds (Obituary, 1941) even though, as he says: 'A few years ago the statement that a type I strain could be changed into a type II or a type III would have been received with greater scepticism than at the present day' (Griffith, 1928). This change in attitude was due, at least in part, to his own studies on bacterial variation.

It seems that the interest of type transformation to Griffith was circumscribed by his concern with epidemiology; having clearly demonstrated the phenomenon he appears not to have attempted to analyse it further, and no further references to it appear among his rather scanty subsequent publications. The fact is that the background of biological knowledge at the time would not, in any case, have held out any obvious clues for further experimental study or even for profitable speculation. Nevertheless, it seems strange, in retrospect, that the most striking and important aspect of transformation as we see it now, namely, that it results in an *inheritable* change of character, is neither mentioned nor implied. However, Griffith did attempt, but failed, to demonstrate transformation in the test tube as well as by means of cell-free extracts, but these experiments were not very rigorous ones. When we consider the stringent requirements later shown to be necessary for reproducible *in vitro* transformation in pneumococci, including the exacting condition of 'competence', the failure of these experiments is not surprising (McCarty, Taylor & Avery, 1946). The nearest Griffith got to an explanation of the phenomenon was a suggestion, based on the comparative thermolability of the transforming capacity of certain (type I) heated suspensions, that it might be mediated, not by the capsular polysaccharide itself, but by 'a specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate' (Griffith, 1928).

I must now, for the moment, leave Griffith's original discovery in order to trace the developments which followed from it. As we shall see, it proved, had he but known it, to be a delayed-action fuse which, 25 years after its publication, triggered off an explosion of biological knowledge, comparable only to that ignited a century ago by the work of Mendel. Following the demonstration that transformation can occur in the test tube (Dawson & Sia, 1931) and can be mediated by cell-free extracts of capsulated pneumococci (Alloway, 1933), O. T. Avery and his colleagues at the Rockefeller Institute undertook a systematic investigation into the chemical nature of the transforming principle. The answer did not come until 1944, but when it did it was a surprising one, for transforming ability turned out to reside in molecules of pure, highly polymerized deoxyribonucleic acid (DNA) (Avery, MacLeod & McCarty, 1944). In addition to the purely chemical evidence was the fact that the activity of transforming preparations resisted completely the action of the enzyme ribonuclease, which attacks ribonucleic acid (RNA), and of proteolytic enzymes, while being rapidly and specifically destroyed by deoxyribonuclease (McCarty & Avery, 1946). Later purification studies (Hotchkiss, 1952) virtually excluded the possibility that transforming activity could be ascribed to molecules of any other substance contaminating the DNA preparations. Alternatives to the idea that DNA was the agent of transformation had become too bizarre to be acceptable.

However, in 1944 the climate of opinion was not favourable to the idea, which the study of transformation had now made explicit, that the genetic material consisted

of DNA. DNA was known to be associated with protein in nuclei and chromosomes, but only proteins had been shown to possess specificity and were considered to have enough structural complexity to carry the innumerable instructions required to specify all the functions of even the simplest cell. The fuse had ignited the priming charge, but the explosion was yet to come. Meanwhile, progress developed along two main lines. One of these was the expanding search for other systems of transformation which revealed that the phenomenon, far from being restricted to pneumococci and the character of capsulation, occurs in many bacterial genera and species, while DNA preparations can transform with respect to virtually any character in which the donor and recipient population differ and whose inheritance by recipient bacteria can easily be recognized (see review by Ravin, 1961).

A second profitable line of inquiry was the study of transformation from the point of view of an exercise in genetic analysis; that is, the outcome of transformation was interpreted in terms of the transfer of fragments of genetic material from a donor to a recipient bacterium, where pairing and genetic exchange, or crossing-over, occurs with the allelic region of the recipient chromosome. In this way a part, or parts, of the recipient chromosome are replaced by allelic donor fragments and recombinant bacteria are generated. Studies of this kind were initiated by Harriet Ephrussi-Taylor (1951), a colleague of Avery, and led to establishment of the following facts which clearly equated the molecules of transforming DNA with fragments of genetic material.

(1) Transformation is a two-way process so that, for example, not only may pneumococci which have lost the ability to produce capsules be transformed to capsulation, but capsulated bacteria can also be transformed to non-capsulation. The difficulty lies only in demonstrating this reciprocity, since only one of the alternative pairs of characters can usually be selected in the way, for example, that capsulated pneumococci were selected by their virulence in Griffith's experiments.

(2) The transformed character is not just added to the sum of the characters of the recipient bacterium but replaces its corresponding, or allelic, character. This is implicit in the reciprocity of transformation which shows that either allele can express itself. If the transformed character were additional, the same transformants would be obtained irrespective of which parental strain was used as donor.

(3) Certain characters, often of a quite different nature, are found to be linked in transformation; that is, bacteria transformed with respect to one of the donor characters turn out to be simultaneously transformed for the other with a fixed probability much higher than can be ascribed to the chance occurrence of two independent transforming events (Hotchkiss & Marmur, 1954). This means that the determinants of the two characters must have a fixed physical relationship to one another so that, in transformation, they are frequently transferred together on the same molecule of DNA. Such physical relationships between character determinants could only be equated with the linkage of genes on chromosome fragments.

(4) Finally, transformation between two strains which are deficient in the *same* character can often lead to restoration of the character. For example, DNA extracted from one non-capsulated strain of pneumococci may transform another non-capsulated strain to normal capsulation. The genetic explanation here, of course, is that mutational lesions affecting different genes mediating polysaccharide syn-

thesis can be made good by genetic exchange, since the two parental bacteria, between them, possess a complete set of good genes.

At this point it may prove interesting to illustrate some of these genetic features by looking afresh at Griffith's original transformation experiments and re-interpreting them in the light of what we know know of the biochemistry and genetics of capsular polysaccharide synthesis. In general, synthesis of the type-specific polysaccharides is mediated by a series of enzymes determined, in turn, by a set of

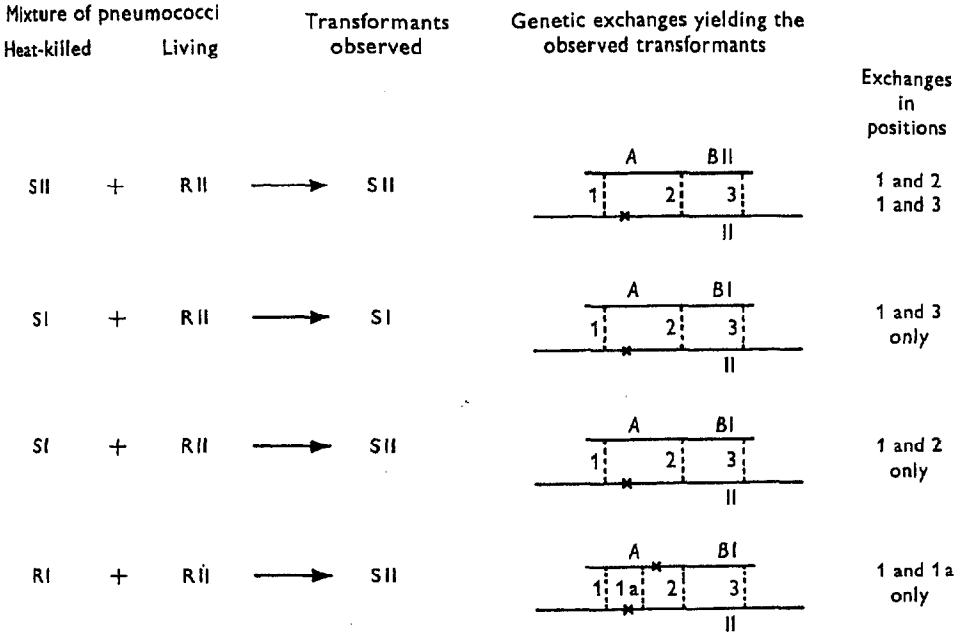

Fig. 1. An interpretation of some of Griffith's transformations of pneumococci in genetical terms. These transformations are recorded in tables VII–XIII of his paper (Griffith, 1928). The pneumococcal strains used in each experiment, and the types of the resulting transformants, are shown on the left of the figure; SI and SII indicate capsulated strains of types I and II respectively, while RI and RII are non-capsulated, rough variants (mutants) of these types. The diagrams on the right show, for each experiment, the positions of genetic exchanges (vertical dotted lines) between recipient chromosome (lower longer line) and donor chromosomal fragment (DNA molecule: upper shorter line), which could yield the observed transformants. The chromosomal regions marked *A* are concerned with that part of the pathway of polysaccharide synthesis common to types I and II capsule; those marked *B* determine the type specificity of the polysaccharide, indicated by the I or II. The site of mutation is shown by –⁎–. The type of transformant depends on the segment of donor fragment which is incorporated into the recipient chromosome by two genetic exchanges. This may be found for any pair of exchanges by tracing along the recipient (lower) chromosome from the left, then up to the donor fragment at the first exchange point and, finally, down again to the recipient chromosome at the second exchange point. See text.

closely linked genes. In the case of a number of pneumococcal types the early steps of the pathway are common to all, so that genetic defects involving them, and leading to failure of capsule production, can be repaired by transforming DNA from another type. On the other hand, the genes determining those later steps in the pathway which confer type-specificity on the polysaccharide, are strict alternatives

which can substitute for one another *en bloc* but cannot participate in mutual repair. It follows that non-capsulated recipients having a mutation blocking the common part of the pathway can be restored to their original type by DNA from donors of a different type; on the contrary, recipients blocked in the specific part of the pathway can be transformed to the donor type only.

On the left of Fig. 1 are shown some of the mixtures of living non-capsulated pneumococci and heat-killed capsulated pneumococci injected into mice by Griffith, and the types of capsulated transformants he observed. It happens that the same non-capsulated strain was used as recipient in all these experiments. Since, as you see, a type II capsule can be restored to this strain by transformation by a type I donor (3rd cross), we may be sure that the mutation leading to non-capsulation involves a gene concerned with that part of the biosynthetic pathway common to both types I and II polysaccharide. The diagrams on the right indicate the positions of genetic exchange between the donor DNA molecules (represented by the shorter upper line of each pair) and the recipient chromosome (the longer lower line). The regions marked '*A*' carry genes which determine biosynthetic steps common to both pathways, the mutation in the recipient being indicated by the cross, while the '*B*' region is concerned with capsular specificity. Note that in transformation, as in other forms of bacterial sexuality, the fragmentary nature of the genetic contribution of the donor demands at least two genetic exchanges, and in any case an even number, to yield a complete recombinant chromosome.

Now let us look at the results. In the first transformation the original capsulation of the recipient can be restored by an exchange in positions 1 and 2 which substitute a functional *A* region for the mutant region, *or* in positions 1 and 3 in which both *A* and *B* donor regions are inherited. In the second transformation the joint inheritance of these *A* and *B* regions is obligatory since not only must the defective *A* region be made good but the ability to synthesize a type I capsule is also conferred. This is, therefore, an example of linked transformation in which at least two, and probably a considerable number of genes, are inherited in a single transformation event. The third transformation, derived from the same mixture as the second, demonstrates the production of different transformant types depending on the position of the second genetic exchange.

Griffith made no comment on the fourth result which must have puzzled him unless he assumed that it was due to a rare reversion. With the advantage of hindsight, however, we now know that it was very much more likely to have resulted from transformation. Unfortunately we have no way of inferring whether the non-capsulated derivative of type I, here used as donor, was defective in region *A* or *B*, but if we assume mutations in the *A* regions of both strains, then the production of capsulated progeny must have resulted from recombination between mutational sites in the same or two very closely linked genes. Thus Griffith, besides carrying out the first genetic crosses in bacteria, may also, however unwittingly, have recorded the first recombination event at the level of what is now termed the genetic fine structure. In any case the discrimination of transformation analysis is inherently quite refined, the scale being set by the comparative size of the donor DNA molecules involved. In systems where the donor DNA is artificially extracted, the molecules usually have a mean molecular weight of about ten million and are long enough to carry some twenty genes. This is about one-hundredth the length of the whole

bacterial chromosome and corresponds approximately to one hundred-thousandth the total chromosomal DNA of a mouse cell.

The increasing assurance which the chemical and genetic study of transformation gave, that the genetic material, at least of bacteria, consisted of DNA, was paralleled by increasingly detailed chemical and physical investigations into the structure of DNA itself. Among the most significant of these investigations were the X-ray diffraction analyses carried out by M. F. H. Wilkins and his colleagues (Wilkins, Stokes & Wilson, 1953; Franklin & Gosling, 1953).

From chemical analysis DNA was known to be a long polymer, composed of repeating molecules of a pentose sugar, deoxyribose, joined together by phosphate molecules. To each sugar molecule is attached any one of four bases—the two purines, adenine and guanine, which are double-ring structures, and the two single-ring pyrimidines, thymine and cytosine. Each unit, consisting of base, sugar and phosphate molecules, is called a nucleotide so that the DNA polymer is a polynucleotide. The X-ray diffraction analyses showed that the polynucleotide chain is in fact arranged as a helix with the bases, which are flat structures, stacked one above the other, and that DNA probably consisted of more than one polynucleotide chain.

Then, early in 1953, just 25 years after the publication of Griffith's discovery came the culmination of this story when Watson & Crick (1953*a*), by a brilliant synthesis, created a model structure for DNA which appeared to satisfy all the data of chemical and diffraction analysis. Time has confirmed the correctness of this structure, whose elucidation was the main explosion which the discovery of transformation, more than any other single event, had first triggered, and whose shock waves still eddy around and disturb the remotest corners of biology.

The elegance and simplicity of this model were too good not to be true, for it at once revealed the nature of those properties of the genetic material which previously had seemed so mysterious; namely, its ability to replicate itself, to carry genetic information, and to undergo inheritable mutation (Watson & Crick, 1953*b*). The model comprises two intertwined polynucleotide helices held together, not by the usual strong co-valent bonds, but by the weak and easily disrupted forces of hydrogen bonding between the bases of the opposing strands which look inwards towards one another. From a biological point of view the most important feature of the model is that, for stereochemical reasons, the hydrogen bonding between the bases of the two helices is highly specific. The regularity of the whole structure requires that adenine bonds only to thymine, and guanine only to cytosine, although there is no restriction whatsoever on the sequence of bases along any one chain. Thus the only irregularity which could carry genetic instructions is the sequence of the four bases, or pairs of bases, along the long axis of the molecule, while accurate transfer of the genetic instructions to the next generation is ensured by the specificity of pairing. If the hydrogen bonds break so that the two polynucleotide strands unwind and separate in a pool of nucleotides, the specific bonding of thymine to adenine and of cytosine to guanine, to reproduce the parental sequence of base-pairs, permits the polymerization of two new strands and the formation of two new daughter duplices identical with the original one. Finally, the mystery of mutation is readily explicable by errors of replication. For example, Watson & Crick (1953*b*) originally pointed out that the specificity of base pairing in their model depends on the

hydrogen atoms of the bases adopting their most stable positions. However, a rare tautomeric shift in the position of a single hydrogen atom of adenine, for example, allows this base to pair with cytosine instead of with thymine; at the next replication the aberrant hydrogen will have reverted to its usual position. On the other hand, the cytosine which was erroneously introduced opposite adenine now pairs with guanine so that, in one of the daughter double helices, an original A–T base-pair has been replaced by a G–C pair; a letter in the genetic code has been permanently altered.

Similarly, the mutagenic action of base analogues, and of many physical and chemical agents which distort the structure of DNA, may be explained in a logical way. I suppose the most dramatic and brilliant achievement to emerge from elucidation of the structure of DNA, has been the solution of the genetic code during the past year, so that virtually all the particular triplets of bases which specify each of the twenty amino acids, as well as two types of punctuation mark, are known (see Stretton, 1965).

I do not intend to digress further into the more recent revelations of molecular biology, which could hardly be regarded as in direct line of descent from the discovery of transformation, although perhaps derived from it in a very ancestral way. Instead, I would like to conclude this lecture by looking at a few of the ways in which transformation has been, and is being, used as a tool in biological research.

Transformation has a twofold application. In the first place it may be used for recombination analysis, and in a number of organisms it may be the only method available. An example of the kind of information it can provide, as well as an example of the way fragmentary inheritance can be a positive asset in certain kinds of study, is an analysis of the mechanism of penicillin-resistance in pneumococci made 15 years ago by Hotchkiss (1951). He found that DNA extracted from a highly resistant donor strain was unable to transform sensitive recipient bacteria to more than a fraction of the donor degree of resistance. However, if a culture of one of these low-degree-resistance transformants was again exposed to the same DNA preparation, transformants showing a higher degree of resistance could be obtained. By repeating this process, sensitive bacteria could be transformed to the donor degree of resistance by a single preparation of donor DNA but in a series of transformation events, each step of this series leading to only a fractional increase in resistance. This type of step-wise inheritance, which characterizes resistance to the majority of antibiotics, is an expression of the fact that high-degree resistance can only be achieved by the summation of a number of independent mutations, usually in unlinked genes; in transformation these genes are carried on separate DNA molecules so that normally only one is taken up at a time by any particular recipient bacterium. In contrast, high degree resistance to streptomycin, for example, is due to mutation in a single gene which probably controls ribosomal structure, and so can be transferred to sensitive recipients by a single transformation event.

Transformation has also been used to great effect in the genetic analysis of *Bacillus subtilis* which is an organism with two very interesting features. In the first place it produces spores and therefore offers what is probably one of the simplest examples of differentiation which, thanks to transformation, is directly accessible to joint biochemical and genetic analysis. Secondly, replication of the chromosome in this organism has a distinctive feature which makes it very suitable for studying

how chromosome division is regulated—a new cycle of replication, following emergence from the stationary phase or from spores, always begins at the same point and proceeds around (or along) the chromosome in the same direction in all the bacilli of a culture. This interesting and important phenomenon was discovered, and then confirmed, by means of two quite different types of transformation experiment.

If we assume that chromosome replication begins at one end of the chromosome, or at a fixed point on a circular chromosome, and runs at uniform speed towards the other, and is continuous, then in the great majority of bacteria of a randomly

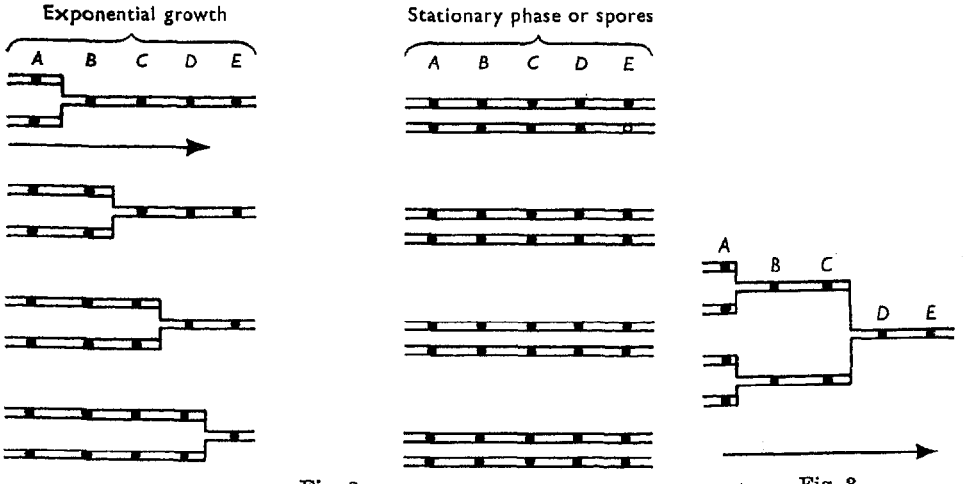

Fig. 2

Fig. 3

Fig. 2. Diagrammatic comparison of the state of chromosome replication among individual *Bacillus subtilis* bacilli in unsynchronized exponentially growing, and stationary-phase populations. The paired lines represent the two polynucleotide strands of the chromosomal DNA. The black circles, designated *A*, *B*, *C*, *D*, *E*, indicate various genes distributed along the chromosome. The arrow indicates the direction of chromosome replication (DNA synthesis), and the fork the position of the growing point of replication. It is assumed that replication begins at a specific point, is polarized and is continuous.

On left: in a randomly growing population during exponential growth, the growing point of replication lies somewhere along the chromosome; in very few bacilli will the chromosome have completed one cycle of replication and not have started another. For every copy of gene *E* there will be two copies of gene *A*, while the number of copies of intermediate genes will lie on a 2:1 gradient in proportion to their distance from *A*.

On right: in a stationary-phase (or spore) population, when growth ceases the current cycle of chromosome replication is completed; a new cycle is initiated only on transfer to a fresh medium.

The figure shows that the ratio of the number of copies of a gene in an exponentially growing population to the number in a stationary-phase population varies from 1·0 close to the initiation point, to 0·5 close to the completion point.

Fig. 3. The diagram explains the relationship between an observed 4:2:1 gene ratio, and initiation of a new replication cycle on the two daughter chromosomes before completion of the initial cycle. The diagram follows upon Fig. 2. See text.

growing population, the growing point at any given moment will lie somewhere between the two extremities, as is shown on the left of Fig. 2 where the black circles, *ABCDE*, represent genes. Very few will have just completed a replication cycle and not yet have started the next. It follows that, in the population, there will be twice

as many copies of a gene located at the starting-point, as at the finishing point. Similarly, the numbers of various intermediate genes should lie on a 2 to 1 gradient depending on the distance of each from the starting point. Clearly the existence of such a gradient could be tested by transformation, on the not unreasonable assumption that the number of transformants with respect to any particular gene is proportional to the number of copies of that gene per unit volume of transforming DNA. However, it happens that different characters may be transformed with very different frequencies for quite other reasons. In order to obtain a true estimate of relative gene numbers, therefore, it is necessary to compare, not the *absolute* numbers of transformants with respect to different genes, but the *ratios* of the transforming capacities of DNA, extracted from exponentially growing cultures on the one hand, and, on the other, from static stationary-phase cultures in which replication of the chromosomes of all the bacteria has been completed so that all the genes are present in equal numbers, as shown on the right of Fig. 2. This ratio has been assessed for 11 genes in *B. subtilis* and the values obtained in fact turn out to lie between 1·0 and 0·5, and to be reproducible (Sueoka & Yoshikawa, 1963). The method thus not only provides evidence for polarized chromosome replication in *B. subtilis*, but also allows the relative locations of the genes along the chromosome to be mapped—an advantage which the fragmentary nature of chromosome transfer normally denies to transformation systems.

A peculiar and unexpected bonus from these studies was the finding that when *Bacillus subtilis* cultures are grown in nutrient broth instead of in a chemically defined medium, the resultant halving of the generation time is accompanied by a change of the 2:1 ratio to a 4:2:1 ratio. As Fig. 3 demonstrates, this seemed to indicate that the chromosome keeps pace with the increased growth rate by initiating a new cycle of replication at the starting point on each of the two daughter chromosomes, at a time when the first cycle is still only half completed. This has since been confirmed (Oishi, Yoshikawa & Sueoka, 1964) and greatly favours the prevalent hypothesis that the pace-maker in the bacterial division cycle is not the nucleus or its equivalent, but the state of the cell membrane which could, of course, be a function of cell mass.

The second type of experiment, which confirmed all the results of the first, illustrates well how transformation can help to establish correlations between physical and genetic data. The donor bacteria are grown up into the stationary phase, or allowed to spore, in a medium rich in the heavy isotopes deuterium and ^{15}N, so that their DNA is denser than normal. In Fig. 4 the two dense strands of the DNA double helix are indicated by the heavy lines. The bacteria, whose chromosomes, as we have seen, are presumptively lined up at the starting-point, are now transferred to a medium containing only light isotopes in which synchronous chromosome replication commences again. As Fig. 4 shows, the newly synthesized DNA has one heavy and one light strand instead of two heavy ones; it is therefore less dense than the parental DNA so that, after extraction of the *total* DNA, the newly synthesized molecular fragments can be cleanly separated from the pre-existing heavy molecules by centrifugation in a density gradient. When this newly synthesized DNA, extracted at intervals throughout the first synchronized generation cycle, is analysed by transformation for the genes it carries, these genes are found to appear in it in a strict and reproducible sequence, indicated by *A*, *B*, *C*, *D*, *E* in the diagram, as

replication of the chromosome proceeds; only at the end of the cycle can the preparation of newly synthesized DNA transform with respect to all the genes (Sueoka & Yoshikawa, 1963; Oishi *et al.* 1964).

Transformation still has a unique and irreplaceable role to play in modern biological research, for it remains the principal method of measuring the biological

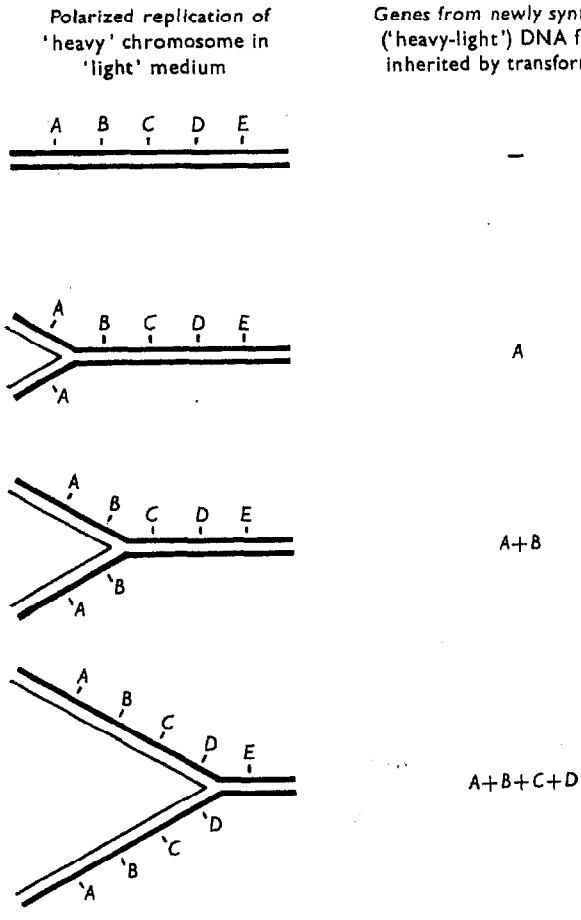

Fig. 4. The diagram illustrates how polarized replication of the chromosome of *Bacillus subtilis* from a specific point may be demonstrated by combined physical and genetic analysis. The heavy lines indicate dense DNA strands which have incorporated ^{2}H and ^{15}N; the light lines are newly synthesized strands of normal density. The diagrams from top to bottom show the progress of DNA synthesis from left to right along the chromosome. The letters, *A–E*, represent a series of genes distributed along the chromosome, whose presence in extracted DNA can be recognized by the transformation of recipient bacilli carrying mutant alleles of these genes. For description of experiment, see text.

activity of DNA. For example, there is little doubt that the ultimate criterion of the *in vitro* synthesis of biologically active DNA from a natural primer will be its transforming ability. Similarly, transformation is a valuable tool in radiation research, or whenever the effects of defined physical or chemical alterations on the biological

activity of DNA must be measured. Thus the phenomenon of photoreactivation, for example (Kelner, 1949), has been found to be due to the action of an enzyme, devoid of species specificity, which combines only with DNA damaged by ultraviolet radiation, requires visible light for its activation, and can restore to u.v.-irradiated transforming DNA a proportion of its biological activity (Rupert, 1961). Without transformation as a meter this enzyme could not have been detected and studied.

In this lecture I have remained loyal to the traditional view of transformation, as a process whereby DNA isolated from a donor strain is able to mediate genetic transfer and recombination between bacteria. But I would like to conclude by extending this concept. The knowledge derived from transformation, that large molecules of nucleic acid, of molecular weight ten million or more, can readily penetrate the walls and semi-permeable membranes of competent bacteria suggested that nucleic acids other than bacterial or, indeed, other than DNA, might similarly gain access to cells. This was first demonstrated for purified ribonucleic acid (RNA) from tobacco mosaic virus which was shown to be infective by itself, though with very low efficiency as compared with the intact virus equivalent, and to promote the synthesis by the plant of new viral RNA and protein and the release of complete infectious viral particles (Gierer & Schramm, 1956; Fraenkel-Conrat, Singer & Williams, 1957). Since then there have been many examples of the infectivity of nucleic acids, from both plant and animal as well as DNA and RNA viruses. More recently, DNA from a *Bacillus subtilis* bacteriophage has been shown to infect competent transformable bacilli of this organism, with the subsequent liberation of normal phage particles—a process for which the name 'transfection' has been coined (Földes & Trautner, 1964). Thus like transformation, viral infection turns out to be a genetic phenomenon.

A remarkable development of these ideas, stimulated partly by recent experimental evidence of the universality of the genetic code, has been the apparently successful attempts to grow animal viruses in transformable bacterial species by exposing competent bacteria to preparations of viral nucleic acids. In this way, complete particles of vaccinia virus have been obtained from *Bacillus subtilis* infected with vaccinia virus DNA, although replication of the viral DNA in the bacteria remains to be proven (Abel & Trautner, 1964). Similarly, by using a special transformation technique, *Escherichia coli* has been infected with RNA from encephalomyocarditis virus, with a resulting formation of complete virus particles (Ben-Gurion & Ginsburg-Tietz, 1965). In this case also there is as yet no evidence of replication of the viral RNA, although it is clear that the RNA can behave as a 'messenger' in *E. coli*, determining the synthesis of specific virus protein. Although it is too early to speculate constructively on the future implications of these astonishing experiments, I hope I have said enough to convince you that, in this twenty-fifth anniversary year of Griffith's death, his most important contribution to knowledge remains as topical and controversial as when he discovered it.

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